



GNE3030R1C10

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Goddard, et al. (as amended)
Appl. No. : 10/035,977
Filed : December 26, 2001
For : NUCLEIC ACIDS ENCODING
POLYPEPTIDES THAT INDUCE CELL
PROLIFERATION (as amended)
Examiner : Daniel E. Kolker
Group Art Unit : 1646

DECLARATION UNDER 37 C.F.R. §1.808

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I hereby aver that the nucleic acid of SEQ ID NO: 56, which encodes the protein of SEQ ID NO: 57, was deposited with the American Type Culture Collection (ATCC) April 20, 1999 and was given ATCC deposit number 203948. Accordingly, the deposited material has been accepted for deposit under the Budapest Treaty on the International Recognition of the deposit of Microorganisms for the Purposes of Patent Procedure and all restrictions on the availability to the public of the material so deposited will be irrevocably removed upon granting of the patent. The deposit will be maintained for a term of at least 30 years and at least five (5) years after the most recent request for the furnishing of a sample of the deposit was received by the depository.

The deposited material is identical to the biological material and was in the Applicant's possession at the time the application was filed.

GENENTECH, INC.

Date: 5/25/05

By: [Signature]

Title: PATENT AGENT



GNE.3030R1C10

PATENT

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Applicant	:	Goddard et al. (as amended)
Appl. No.	:	10/035,977
Filed	:	December 26, 2001
For	:	NUCLEIC ACIDS ENCODING POLYPEPTIDES THAT INDUCE CELL PROLIFERATION (as amended)
Examiner	:	Kolker, Daniel E.
Group Art Unit	:	1646

DECLARATION UNDER 37 CFR §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

We declare and state as follows:

1. We are the inventors of the invention claimed in the above-captioned patent application.
2. During the time period in which we participated in the events and activities described herein, we were employed by Genentech, Inc., the assignee of the above-captioned application.
3. All of the events and activities described herein were performed by us personally, or by others at our direction as part of our duties as employees of Genentech, Inc.
4. The invention claimed in the above-captioned patent application was conceived and reduced to practice in the United States prior to November 18, 1999 as described below.
5. Prior to November 18, 1999, we conceived of the invention claimed in the above-captioned patent application. This is demonstrated by the attached sequence printout (Exhibit A), which was generated prior to November 18, 1999, and which shows the complete sequence of the nucleic acid having the sequence of SEQ ID NO: 56. The attached printout also shows the complete sequence of the polypeptide which has the sequence of SEQ ID NO: 57. As evidenced by the sequence printout, we were in possession of the complete nucleic acid and amino acid sequences prior to November 18, 1999.
6. The date deleted from Exhibit A is prior to November 18, 1999. This date was redacted pursuant to M.P.E.P. § 715.07. The date that remains is the date the report was printed, April 28, 2005.

Appl. No. : 10/035,977
Filed : December 26, 2001

7. After these initial experiments, we diligently reduced the claimed subject matter to practice by working to express and purify the encoded polypeptide and to run it systematically through many assays. The cDNA was deposited with the American Type Culture Collection (ATCC) on April 20, 1999 and assigned ATCC no. 203948. The protein of interest was assigned a "protein inventory number" (e.g., PIN1205-1), and this protein is a polypeptide having the sequence of SEQ ID NO:57, and is encoded by SEQ ID NO: 56.

8. Exhibit B shows that the protein lot designated PIN1205-1 was delivered to James Pan on a date prior to November 18, 1999 in order to perform assay ASY92, called "Mouse Mesangial Cell proliferation Assay." Also, as shown in Exhibit B, the assay was completed on a date prior to November 18, 1999. Exhibit B also shows that the tested polypeptides tested positive ("All Positives"), thereby confirming the ability of the encoded polypeptide to induce mesangial cell proliferation. Thus, actual reduction to practice occurred on a date prior to November 18, 1999.

9. The dates deleted from Exhibit B all are prior to November 18, 1999. These dates were redacted pursuant to M.P.E.P. § 715.07. The date that remains is the date the report was printed, April 28, 2005.

10. After reducing the invention to practice, we worked with the Genentech, Inc. patent department to prepare a non-provisional patent application, which included the sequences of SEQ ID NO:56 and SEQ ID NO:57, as well as the data showing the ability to induce mesangial cell proliferation. That application was filed on March 1, 2000 as PCT/US00/05601.

11. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

By: _____
Audrey Goddard


Date: _____

By: _____
Paul J. Godowski

Date: _____

By: _____
Austin L. Gurney

Date: _____

By: _____

James Pan

Date: June 9/05

By: _____
Colin K. Watanabe

Date: _____

By: _____
William I. Wood

Date: _____

Appl. No.
Filed



10/035,977
December 26, 2001

7. After these initial experiments, we diligently reduced the claimed subject matter to practice by working to express and purify the encoded polypeptide and to run it systematically through many assays. The cDNA was deposited with the American Type Culture Collection (ATCC) on April 20, 1999 and assigned ATCC no. 203948. The protein of interest was assigned a "protein inventory number" (e.g., PIN1205-1), and this protein is a polypeptide having the sequence of SEQ ID NO:57, and is encoded by SEQ ID NO: 56.

8. Exhibit B shows that the protein lot designated PIN1205-1 was delivered to James Pan on a date prior to November 18, 1999 in order to perform assay ASY92, called "Mouse Mesangial Cell proliferation Assay." Also, as shown in Exhibit B, the assay was completed on a date prior to November 18, 1999. Exhibit B also shows that the tested polypeptides tested positive ("All Positives"), thereby confirming the ability of the encoded polypeptide to induce mesangial cell proliferation. Thus, actual reduction to practice occurred on a date prior to November 18, 1999.

9. The dates deleted from Exhibit B all are prior to November 18, 1999. These dates were redacted pursuant to M.P.E.P. § 715.07. The date that remains is the date the report was printed, April 28, 2005.

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11. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

By: _____
Audrey Goddard

Date: _____

By: _____
Paul J. Godowski

Date: _____

By: _____
Austin L. Gurney

Date: 6/8/05

By: _____
James Pan

Date: _____

By: _____
Colin K. Watanabe

Date: _____

By: _____
William I. Wood

Date: _____

EXHIBIT A

GENE/GENES

SEQUENCE VIEWER

SEQUENCE VIEWER

GENE MAP

GENE MAP

GENE MAP

Find C New C Update

Find C New C Update

Find C New C Update

Additional Resources

Additional Resources

Additional Resources

ASY92		Mouse Mesangial Cell Proliferation Assay	
Assay Name	Mouse Mesangial Cell Proliferation Assay	Alias Name	Mu Mess Cell Prolif
Status	Retired	Class	Primary
Format	96 Well	Type	Cell
Sample Requirements	Screen SPDI proteins which can stimulate Mesangial Cell Proliferation		
Assay Volume	0.1 ml	Fold Dil Into Well	10 Fold
Replicates	3	Dilutions	2
Volume Requested	0.03ml/well/conc	Species	Mouse
Protocol	Screen SPDI proteins which can stimulate Mesangial Cell Proliferation		
Purpose	On day 1: Mouse mesangial cells are plated on a 96 well plate in Media(A 3:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium- 95%- fetal bovine serum-5%- supplemented with 14mM hepes) and grw overnight. On day 2: SPDI Proteins are diluted at 2 conc. (1%- 0.1%) in serum-free Media and added to the cells. On day 4: After 48 hours incubation- each well of the plate was added 20 µl of the Cell Titer 96 Aqueous one solution reagent (Promega) and colorimetric reaction was allowed for 2 hours. The absorbance [OD] is measured at 490 nm.		
Matrix	Promega kit for the assay-	Result Calculation	replicated average
Result Interpretation	Any PIN that gives an absorbance reading which is 15% above the media control is considered a hit.	Result Cutoff	> 15 %
In Vivo: In Vitro:			
Comments			
Status	Retired	Cancel Reason	Endocrinology
Data Entered		Bioreas	Endocrinology
Data Cancelled		Lab Scientist	Wenguang Mao
Department	Endocrinology		
Scientist	James (Guohua) Pan		
Notebook	0.		
Assays			
ASY DNA DOM EXP FAM FLS LUB LOT MAP QLI PER PRD PUR RNA SRC UNQ XET YST			
ASSAY VIEWER Sequence Viewer Gene Viewer GeneGenes RAISE			
GeneGenes Feedback			

GENECHOICES

SEARCH

Find C New C Update

SELECT

SEQUENCE VIEWER

ASSAY VIEWER

GENECHOICES

SEARCH

Find C New C Update

SELECT

SEQUENCE VIEWER

ASSAY VIEWER

SFDI Assays

Assay Viewer

Find Lots

Find PIN

Find DNA

Show Lots for:

PIN: 1205

Number: 1205

Include UNQ Related Lots

Find PIN

Find DNA

Show Lots for:

PIN: 1205

Number: 1205

Include UNQ Related Lots

Lots for Search

Find PIN

Find DNA

Show Lots for:

PIN: 1205

Number: 1205

Include UNQ Related Lots

☐ All Positives ☐ Verified Positives ☐ Pending

Date Complete From To

ASSAY RESULT LIST

ASY	ASY Name	PUR/EXP/DNA	LOT	LOT Name	Pos	Verified	Score	Cons Unit	Mean	Cnt	UNQ	Protein Name	Comment
ASY92	Mu Mess Cell Prolif	PUR1715	LOT2447	PIN1205-1			0.10	%	1	1	UNQ1815	Human DPKL1915 IgG	
ASY92	Mu Mess Cell Prolif	PUR1715	LOT2447	PIN1205-1			1.00	%	1	1	UNQ1815	Human DPKL1915 IgG	

ASY | DNA | DOM | EXP | FAM | FLS | LIB | LOT | MAP | QLI | PBB | PRO | PUR | RNA | SRC | UNQ | XET | YST

Assay Viewer | Sequence Viewer | Gene Viewer | GeneGenes | SAGE

GenoGen Feedback

EXHIBIT B


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>Thursday, April 28, 2005
>DNA92234 [Full]
>887 Sites [All Sites]
> [DNA92234], sheldens
> Lib309
>Sequence confirmed by phredphrap

      thai
      nlaIII  snaBI
      sphI  fnuDII/mvnI      mnII
      nspHI bstUI taiI      taqI
      taiI nspI bsh1236I      xhoI
      maeII/hpyCH4IV bsiWI/splI      tsp509I[M.ecoRI-]
      aluI hinII/acyI cac8I bsaAI      ecoRI      tliI
      sapi  ahaiI/bsaHI mlul rsai      hpy188I      smII
      mboII  aatII cac8I aflIII maeII/hpyCH4IV      paer7I      hpy188I aciI      bpmI/g
      hphI  sfcI earI/ksp632I hpy99I hpyCH4V csp6I aluI apoI      aval[M.taqI-]      mnII      fnu4HI/bsaFI hpy18
1 TAGGTGACAC TATAGAAGAG CTATGACGTC GCATGCACGC GTACGTAAGC TCGGAATTGC GCTCGAGGAA TGAATACCTC CGAAGCCGCT TTGTTCTCCA
ATCCACTGTG ATATCTTCTC GATACTGCAG CGTAGCTGCG CATGCATTGC AGCCTTAAGC CGAGCTCCTT ACTTATGGAG GCTTCGGCGA AACAGAGGT
^insert starts here

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ncII
mspI
hpaII
dsaV
bpuAI bssKI bsp1286
bbsI bslI bsmFI taiI bmyI
aluI mnlI mboII bsaJI maeII/hpyCH4IV msel maeIII nla
101 GATGTGAATA GCTCCACTAT ACCAGCCTCG TCTTCTCTCC GGGGACAAC GTGGGTCAGG GCACAGAGAG ATATTTAATG TCACCTCTT GGGGCTTTCA
CTACACTTAT CGAGGTGATA TGGTCGGAGC AGAAGGAAGG CCCCCTGTTG CACCAGTCC CGTGTCTCTC TATAAATTAC AGTGGGAGAA CCCCAGAACT

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sau3AI
mboI/ndeII[dam-]
dpnII[dam-]
dpmI[dam+]
alwI[dam-]
nlaIV

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pleI mlyI hinfI bsmFI mnlI
201 TGGGACTCCC TCTGCCACAT TTTTGGAGG TTGGGAAAGT TGCTAGAGGC TTCAGAACTC CAGCCTAATG GATCCCAAAC TCAGGAGAAT GGCTGCGTCC
ACCTGAGGG AGACGGTGTA AAAAACCTCC AACCTTTCA AGCTCTCCG AGCTCTGAG GTCGGATTAC CTAGGTTTG AGCCTCTTA CCGACGCGAGG

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M D P K L G R M A A S
^MET

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[illegible]

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pspGI sau96I[M.haeIII-]
mvaI pspOMI/bsp120I
ecoRII[dcM-]
dsaV[dcM-]
bstNI nlaIV
bssKI[dcM-]
hinPI bsp1286[M.haeIII-]
hhaI/cfoI sfiI
tseI bsaJI bmyI
fnu4HI/bsoFI sau96I[M.haeIII-]
bbvI apyI[dcM+]
dsal tseI hpyCH4V banII[M.haeIII-]
btgI/bstDSI sfcI haeII apaI mnlI
bsaJI aciI tseI alwNI[dcM-] haeIII/palI bsaJI
mwOI fnu4HI/bsoFI pstI[M.HI-] nlaIV haeIII/palI
bceAI bbvI fnu4HI/bsoFI eco0109I/draII nlaIII mnlI bbvI
haeIII/palI bbvI alw26I/bsmAI bgII[M.haeIII-] pshAI avaII alw26I/bsmAI hpy188I mnlI
501 GGCCGTGGCT GCGGACACGC TGCAGCGCCT GGGGGCCCGT GTGGCCTCGG TGGACATGGG TCCTCAGCAG CTGCCCCGATG GTCAGAGTCT TCCAATACCT
CCGGCACCGA CGCCTGTGCG ACGTCGCGGA CCCCCGGGCA CACCGGAGCC ACCTGTACCC AGGAGTCGTC GACGGGCTAC CAGTCTCAGA AGGTTATGGA
79 A V A A D T L Q R L G A R V A S V D M G P Q Q L P D G Q S L P I P

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scrFI[dcM-]
    pspGI
    mvaI
    ecoRII[dcM-]
    dsav[dcM-]
    bstNI bslI
    bssKI[dcM-]
    apyI[dcM+]
    fokI cfrI bsrI
    bstF5I haeIII/palI
    601 CCCGTCATCC TGGCCGAAC T GGGAGCGAT CCCACGAAAG GCACCGTGTG CTTCTACGGC CACTTGGACG TGCAGCCTGC TGACCGGGGC GATGGGTGGC
    GGGCAGTAGG ACCGGCTTGA CCCCTCGCTA GGTGCTTTC CGTGGCACAC GAAGATGCCG GTGAACCTGC ACGTCGGACG ACTGGGCCCG CTACCCACCG
    112 P V I L A E L G S D P T K G T V C F Y G H L D V Q P A D R G D G W L

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    mvaI
    ecoRII[dcM-]
    dsav[dcM-]
    bstNI bslI
    bssKI[dcM-]
    apyI[dcM+]
    fokI cfrI bsrI
    bstF5I haeIII/palI
    601 CCCGTCATCC TGGCCGAAC T GGGAGCGAT CCCACGAAAG GCACCGTGTG CTTCTACGGC CACTTGGACG TGCAGCCTGC TGACCGGGGC GATGGGTGGC
    GGGCAGTAGG ACCGGCTTGA CCCCTCGCTA GGTGCTTTC CGTGGCACAC GAAGATGCCG GTGAACCTGC ACGTCGGACG ACTGGGCCCG CTACCCACCG
    112 P V I L A E L G S D P T K G T V C F Y G H L D V Q P A D R G D G W L

    sau96I
    nlaIV
    avaiI
    701 TCACGGACCC CTATGTGCTG ACGGAGTAG ACGGGAAC T TATGACGA GGAGCGACCG ACAACAAAGG CCCTGTCTTG GCTTGGATCA ATGCTGTGAG
    AGTGCCTGGG GATACACGAC TGCCTCCATC TGCCTTTGA AATACCTGCT CCTCGCTGGC TGTTGTTTCC GGGACAGAAC CGAACCTAGT TACGACACTC
    146 T D P Y V L T E V D G K L Y G R G A T D N K G P V L A W I N A V S

    sau3AI mwoI
    bslI
    sau96I[M.haeIII-]
    haeIII/palI
    eco0109I/draII
    alwI[dam-]
    hae
    701 TCACGGACCC CTATGTGCTG ACGGAGTAG ACGGGAAC T TATGACGA GGAGCGACCG ACAACAAAGG CCCTGTCTTG GCTTGGATCA ATGCTGTGAG
    AGTGCCTGGG GATACACGAC TGCCTCCATC TGCCTTTGA AATACCTGCT CCTCGCTGGC TGTTGTTTCC GGGACAGAAC CGAACCTAGT TACGACACTC
    146 T D P Y V L T E V D G K L Y G R G A T D N K G P V L A W I N A V S

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ecorII[dcn-]     pspGI
dsav[dcn-]       mvaI
bstNI            ecorII[dcn-]
bspl286          dsav[dcn-]
bmyI bssKI[dcn-] bstNI
hpy188I apyI[dcn+] dpnI[dcn+]
eco57I bsaJI    bglII
mwoI banII bpmI/gsuI[dcn-]
801 CGCCTTCAGA GCCTGGAGC AGATCTTCC TGGAATATC AAATCATCA TTGAGGGGAT GGAAGAGGCT GGCTCTGTG CCCTGGAGGA ACTGTGGAA
    GCGGAAGTCT CCGGACCTCG TTCTAGAAGG ACACCTTATAG TTTAAGTAGT AACTCCCTTA CCTTCTCCGA CCGAGACAAAC GGGACCTCCT TGAACACCTT
179 A F R A L E Q D L P V N I K F I I E G M E E A G S V A L E E L V E

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hpall
dsav
bsKI
bsaJI
xmaI/ps
smaI
scrFI[M
ncII
dsav
bsKI
bsaJI
avaI[M.
nlaIV
sau3AI
mboI/ndeII[dam-]
dpnII[dam-]
dpnI[dam+]
alwI[dam-]
cac8I
hpy188I
tsp509I
sau96I mboII
avaII hinfI
901 AAAGAAAAGG ACCGATTCTT CTCTGGTGTG GACTACATTG TAATTTCAGA TAACCTGTGG ATCAGCCAAA GGAAGCCAGC AATCACTTAT GGAACCCGGG
TTTCTTTTCC TGGCTAAGAA GAGACACAC CTGATGTAAC ATTAAGTCT ATTGGACACC TAGTCGGTTT CCTTCGGTCG TTACTGAATA CCTTGGGCC
212 K E K D R F F S G V D Y I V I S D N L W I S Q R K P A I T Y G T R G

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mvaI
ecoRII[dcM-]
dsaV[dcM-]
bstNI
bsaKI[dcM-]
bsmAI
hphI
alul
nlaiIII
mnli
hpyCH4V
apyI[dcM+]
bsaI
ddei
nlaiV
hpy188III
fokI
rcal
bstF5I
hpy188III
sfal
bspHI
sau3AI
sap
mboI/ndeII[da
dpnII[dam-]
dpnI[dam+]
ea
1001 GGAACAGCTA CTTCAATGGTG GAGGTGAAAT GCAGAGACCA GGATTTTCAC TCAGGAACCT TTGGTGGCAT CCTTCATGAA CCAATGGCTG ATCTGGTTGC
CCTTGTCGAT GAAGTACCAC CTCCACTTTA CGTCTCTGGT CCTAAAAGTG AGTCCTTGA AACCCCGTA GGAAGTACIT GGTACCGAC TAGACCAACG
246 N S Y F M V E V K C R D Q D F H S G T F G G I L H E P M A D L V A

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scrFI[dcn-]
pspGI
mvaI
ecoRII[dcn-]
dsav[dcn-]
bstNI
bssKI[dcn-]
sau96I[dcn-]
nlaIV
avaII[dcn-]
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pspGI apyI[dcn+]
mvaI bsmFI
ecoRII[dcn-]
dsav[dcn-]
bstNI bsaJI
bssKI[dcn-] tfII
apyI[dcn+] hinfI
xmnI nlaIV mboII
asp700 mnlI earI/ksp632I
1101 TCTTCTCGGT AGCCTGGTAG ACTCGTCTGG TCATATCTTG GTCCCTGGAA TCTATGATGA AGTGGTTCCT CTTACAGAAG AGGAATATAA TACATACAAA
AGAAGAGCCA TCGGACCATC TGAGCAGACC AGTATAGGAC CAGGGACCTT AGATACTACT TCACCAAGGA GAATGTCTTC TCCTTTATTT ATGTATGTTT
279 L L G S L V D S S G H I L V P G I Y D E V V P L T E E E I N T Y K

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rsal
csp6I
nlaIV
kpnI
bani
asp7I8
bpmI/gsuI[dcM
hpy188III
acc65I
mnlI
hpyCH4V
mnlI
1201 GCCATCCATC TAGACCTAGA AGAATACCGG AATAGCAGCC GGGTTGAGAA ATTCTGTTC GATACTAAGG AGGAGATTCT AATGCACCTC TGGAGGTACC
CGGTAGTAG ATCTGGATCT TCTTATGGCC TTATCGTCGG CCCAACTCTT TAAAGACAAG CTATGATTCC TCCTCTAAGA TTACGTGGAG ACCTCCATGG
312 A I H L D L E E Y R N S S R V E K F L F D T K E E I L M H L W R Y P

haeIII/pali
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cfri
scrFI[dcM-]
pspGI
mval
ecoRII[dcM-]
dsav[dcM-]
bstNI
tsp509I
bsm
rmaI
maeI
bfai
1301 CATCTCTTTC TATTCATGG ATCGAGGGCG CGTTTGATGA GCCTGGAACT AAAACAGTCA TACCTGGCGG AGTTATAGGA AAATTTCAA TCCGTCTACT
GTAGAGAAAG ATAAGTACCC TAGCTCCCGC GCAAACTACT CGGACCTTGA TTTTGTCACT ATGGACCGGC TCAATATCCT TTTAAAAGTT AGGCAGATCA
346 S I S I H G I E G A F D E P G T K T V I P G R V I G K F S I R L V

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[illegible]

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sau3AI
mboI/ndeII[dam-]
dpnII[dam-]
fokI dpnI[dam+]
bstF5I
scrFI[M.hpaII-]
ncII alwI[dam-]
mspi nlaIV
hpaII bstYI/xhoII
dsav bamHI tsp509I muni/mfeI
bssKI alwI[dam-] muni/mfeI
1601 TCCGGGATGG ATCCACCAAT CCAATTGCCA AAATGTTCCA GGAGATCGTC CACAAGAGCG TGGTCTTAAT TCCGCTGGGA GCTGTGTATG ATGAGAACA
AGGCCCTACC TAGGTGGTAA GGTTAACGGT TTTACAAGGT CCTCTAGCAG GTGTTCTCGC ACCACGATTA AGGCGACCGT CGACAACACTAC TACCTCTTGT
446 R D G S T I P I A K M F Q E I V H K S V V L I P L G A V D D G E H

sau3AI
scrFI[dcn-]
pspGI mboI/ndeII[dam-]
mvaI dpnII[dam-]
ecorIII[dcn-]
dsav[dcn-]
bstNI dpnI[dam+]
bssKI[dcn-]
apyI[dcn+] mwoI aciI aluI
tsp509I
mspAI/nspBII
tru9I
tseI aluI mseI
nlaIV fnu4HI/bsoFI sau96I[M.haeIII-]
mnlI tsp509I bbvI ddeI haeIII/palI aseI/asnI/vspI
1701 TTCGCAGAT GAGAAAATCA ACAGGTGGAA CTACATAGAG GGAACCAAT TATTGCTGC CTTTCTTCTTA GAGATGGCCC AGCTCCATTA ATCACAAGAA
AAGCGTCTTA CTCTTTTAGT TGTCCACCTT GATGTATCTC CCTTGCTTTA ATAAACGACG GAAAAGAAT CTCTACCGGG TCGAGGTAAT TAGTGTCTT
479 S Q N E K I N R W N Y I E G T K L F A A F F L E M A Q L H O

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sau3AI
mboI/ndeII[dam-]
dpnII[dam-]
dpnI[dam+]
hpy188I
sau3AI tspRI
hpy188I alwI[dam-]
rmaI mboI/ndeII[dam-] hphI
maeI dpnII[dam-] tfII mnlI foki bfaI foki
bfaI dpnI[dam+] hinfI[M.hphI-] bstF5I bstF5I
1801 CCTTCTAGTC TGATCTGATC CACTGACAGA TTCACCTCCC CCACATCCC AGACAGGGAT GGAATGTAAA TATCCAGAGA ATTTGGGTCT AGTATAGTAC
GGAAGATCAG ACTAGACTAG GTGACTGTCT AAGTGGAGGG GGTTAGGGA TCTGTCCCTA CCTTACATTT ATAGGTCTCT TAAACCCAGA TCATATCATG

sau96I
nlaIV
avaII hpyCH4V
ppuMI bsgI
ecoO109I/draII
tru9I tspRI
mseI bsmFI btsI
sau3AI
mboI/ndeII[dam-]
dpnII[dam-]
dpnI[dam+]
hpy188III
msei
ahaII/draI ecorV alwI[dam-] sspI
1901 ATTTTCCCTT CCATTTAAAA TGCTTTGGGA TATCTGGATC AGTAATAAAA TATTTCAAAG GCACAGATGT TGGAAATGGT TTAAGGTCCC CCACTGCACA
TAAAGGGAA GGTAATTTT ACAGAACCCCT ATAGACCTAG TCATTATTTT ATAAAGTTTC CGTGTCTACA ACCTTTACCA AATCCAGGG GGTGACGTGT

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scrFI[dcM-]
pspGI
mvaI
ecoRII[dcM-]
dsaV[dcM-]
bstNI
bssKI[dcM-]
apyI[dcM+]
bslI      tfil
hpyCH4V   bsaJI   hinfi
2001 CCTTCCTCAA GTCATAGCTG CTTGATTTCC CCAAGTCCTG TGAATAGCC CCAGGATTGG ATTCCTTCCA ACCTTTTCAGC ATATCTCCAA
GGAAGGAGTT CAGTATCGAC GAACGTCGTT GAACTAAAGG GGTTCAGGAC ACGTTATCGG GGTCTTAACC TAAGGAAGGT TGGAAAATCG TATAGAGGTT

tseI
cac8I
tseI   fnu4HI/bsoFI
fnu4HI/bsoFI
bbsI   bbsI
aluI   hpyCH4V
mslI   mslI
mnlI   mnlI
mspI   mspI
hpaII  hpaII
bsaWI  bsaWI
tsp509I
hpyCH4V
2101 CCTTGCATTT TGATTGGCAT AATCACTCCG GTTGTCTTTC TAGTCTCTCA AGTGCTCGTG ACACATAATC ATTCCATCCA ATGATCGCCT TTGCTTTACC
GGAACGTTAA ACTAACCGTA TTAGTGAGGC CAAACGAAAG ATCCAGGAGT TCACGAGCAC TGTGATTTAG TAAGGTAGGT TACTAGCGGA AACGAAATGG

trui
mseI
aseI/asnI/vspI   bsaI   tspRI
2201 ACTCTTTTCT TTTATCTTAT TAATAAAAAT GTTGGTCTCC ACCACTGNCT CCCAAAAAAA AAAAAAAA AAAAATAAAA AAAAAAAA
TGAGAAAGGA AATAGAATA ATTATTTTA CAACGAGG TGGTGACNGA GGGTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT

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scrFI[M.hpaII-]
ncII
mspI
hpaII
dsav
bssKI          sau96I rsaI
xmaI/pspAI     rsrII/cspI
smaI           mroI   nlaIV
acII           scrFI[M.hpaII-] cpoI kpnI hpyCH4V
fnu4HI/bsoFI   tagI ncII      hpy188III csp6I
haeIII/palI    sstI salI dsav   bspMII  banI sfcI
mcRI           sacI hincII/hindII[M.taqI-] avaiI[M.hpaII-]
eagI/xmaIII/eclXI aluI accI[M.taqI-] tru9I mspI  asp718
eaeI           hgiAI/aspHI[M.aluI-] mseI bspEI cfr10I/bsrFI
cfrI           rmaI  ecl136II  bssKI  aseI/asnI/vspI  acc65I  cac8I
bsiEI          maeI  bsp1286[M.aluI-] xmnI  tsp509I  bsaWI  pstI
notI           bfai  bsiHKAI   bsaJI  tsp509I  bsaWI  ageI   sse8387I
fnu4HI/bsoFI   bmyI hpy99I avai[M.hpaII-] hpaII mspI bspMI   rsaI
acII           speI  banII[M.aluI-]  asp700  accIII hpaII sbfI   csp6I aluI  sf
2301 AAAAAAAAAA AAAGGCGGC CGCCGACTAG TGAGCTCGTC GACCCGGGAA TTAATTCCGG ACCGGTACCT GCAGGCGTAC CAGCTTTTCCC
TTTTTTTTTT TTTTTTTTTT TTTCCCGCCG GCGGCTGATC ACTCGAGCAG CTGGGCCCTT AATTAAGGCC TGGCCATGGA CGTCCGCATG GTCGAAAGGG
pleI
mlyI
hinFI          aluI
2401 TATAGTGAGT CGTATTAGAG CTTGG
ATATCACTCA GCATAATCTC GAACC

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> length: 2425

aatII (GACGTC) :	25	
acc65I (GGTACC) :	1295	2374
accI (GTMKAC) :	727	1117 2348
accIII (TCCGGA) :	2366	
aciI (CCGC) :	86	332 355 511 1420 1672 2326 2330
acyI (GRCGYC) :	25	
aflIII (ACRYGT) :	37	
ageI (ACCGGT) :	2371	
ahaII (GRCGYC) :	25	
ahaIII (TTTAAA) :	1914	
aluI (AGCT) :	19	48 110 485 569 1006 1680 1781 2016 2343 2392 2419
alw26I (CAGNNNCTG) :	418	523 565
alwI (GGATCNNNN) :	270	271 628 785 959 1319 1599 1609 1610 1817 1936
alwNI (CAGNNNCTG) :	418	523 565
apaI (GGGCC) :	533	
apoI (RAATTY) :	54	409 841 1249 1381 1879
apyI (CCWGG) :	528	609 813 882 1038 1113 1137 1144 1342 1363 1638 2061
aseI (ATTAAT) :	1787	2219 2360
asnI (ATTAAT) :	1787	2219 2360
asp700 (GAANNNTTC) :	375	1159 1379 1469 2358
asp718 (GGTACC) :	1295	2374
asphi (GWGCWC) :	484	2152 2342
aspi (GACNNNGTC) :	451	
avaI (CYCGRG) :	62	280 995 2353
avaII (GGWCC) :	559	705 909 1140 1985 2143 2369
balI (TGGCCA) :	437	
bamHI (GGATCC) :	270	1609
banI (GGYRCC) :	640	1295 2374

banII (GRGTC) : 484 533 809 2342
 bbsI (GAAGACNNNNN) : 130 379 587
 bbvI (GCAGC) : 292 312 315 318 321 508 519 522 567 570 672 1235 1552 1756 2017 2024
 bceAI (ACGGCNNNNNNNNNN) : 502 656
 bfaI (CTAG) : 243 1210 1216 1396 1504 1805 1849 1889 2140 2337
 bglI (GCCNNNNNGGC) : 535
 bglII (AGATCT) : 822
 bmyI (GDGCHC) : 159 484 533 809 2152 2342
 bpmI (CTGGAG) : 96 258 325 814 883 1290
 bpuAI (GAAGACNNNNNN) : 130 379 587
 bsaAI (YACGTR) : 42
 bsaHI (GRCGYC) : 25
 bsaI (GGTCTCNNNN) : 1034 2234
 bsaJI (CCNNGG) : 139 359 503 528 545 684 812 881 995 996 1143 1516 2060 2353
 bsaWI (WCCGGW) : 1226 2127 2366 2371
 bseRI (GAGGAGNNNNNNNN) : 342 749 1270
 bsgI (GTGCAG) : 415 670 1994
 bsh1236I (CGCG) : 38 331 1329
 bsiEI (CGRYCG) : 755 2327
 bsiHKAI (GWGCWC) : 484 2152 2342
 bsiWI (CGTACG) : 40
 bsli (CCNNNNNNNGG) : 135 184 274 275 354 396 614 631 771 1847 1848 2060
 bsmAI (GTCTC) : 1034 2235
 bsmAI (GTCTC) : 1034 2235
 bsmFI (GGGACNNNNNNNNNN) : 143 202 297 1141 1399 1986
 bsoFI (GCNGC) : 85 292 312 315 318 321 332 508 519 522 567 570 672 1235 1552 1756
 2017 2024 2326 2329
 bsp120I (GGGCC) : 533
 bsp1286 (GDGCHC) : 159 484 533 809 2152 2342
 bspCNI (CTCAGNNNNNNNN) : 563 1050

bspEI (TCCGGA) : 2366
 bspHI (TCATGA) : 1074
 bspMI (ACCTGC) : 2377
 bspMII (TCCGGA) : 2366
 bsrFI (RCCGGY) : 2371
 bsrI (ACTGGN) : 384 618 1542
 bssKI (CCNGG) : 139 360 528 609 684 813 882 995 996 1038 1113 1137 1144 1239 1342
 1363 1602 1638 2061 2353 2354
 2155
 bssSI (CTCGTG) : 643 1354 1573
 bst4CI (ACNGT) : 641
 bstAPI (GCANNNNTGCG) : 503 1516
 bstDSI (CCRYGG) : 405 606 857 1068 1203 1605 1844 1857 2175
 bstFSI (GGATG) : 528 609 813 882 1038 1113 1137 1144 1342 1363 1638 2061
 bstNI (CCWGG) : 38 331 1329
 bstUI (CGCG) : 260 1478
 bstXI (CCANNNNTTGG) : 270 822 1609
 bstYI (RGATCY) : 503 1516
 btgI (CCRYGG) : 667
 btrI (CAGTC) : 1992
 btsI (GCAGTGNN) : 31 35 303 675 868 975 2020 2381
 cac8I (GCNNGC) : 330 364 525 800 1328
 cfoI (GCGC) : 2371
 cfr10I (RCCGGY) : 437 500 611 657 1365 2327
 cfrI (YGGCCR) : 2368
 cpoI (CGGWCCG) : 41 387 1296 1897 2375 2387
 csp6I (GTAC) : 2368
 cspI (CGGWCCG) : 563 1050 1265 1767
 ddeI (CTNAG) : 271 628 786 823 960 1090 1320 1566 1599 1610 1644 1812 1817 1937
 dpnI (GATC) : 2183

dpaII (GATC): 271 628 786 823 960 1090 1320 1566 1599 1610 1644 1812 1817 1937
 2183
 draI (TTTAAA): 1914
 draII (RGGNCCY): 532 558 768 1984 2142
 draIII (CACNNNGTG): 642
 dsaI (CCRYGG): 503 1516
 dsaV (CCNGG): 139 360 528 609 684 813 882 995 996 1038 1113 1137 1144 1239 1342
 1363 1602 1638 2061 2353 2354
 eaeI (YGGCCR): 437 500 611 657 1365 2327
 2327
 eagI (CGGCCG): 15 487 862 1100 1177
 earI (CTCTTCNNNN): 484 2342
 ecl136II (GAGCTC): 2327
 eclXI (CGGCCG): 250 424 474 489 804
 eco57I (CTGAAG): 396
 ecoNI (CCTNNNNNAGG): 532 558 768 1984 2142
 ecoO109I (RGGNCCY): 54
 ecorI (GAATTC): 528 609 813 882 1038 1113 1137 1144 1342 1363 1638 2061
 ecorII (CCWGG): 1929
 ecorV (GATATC): 85 292 312 315 318 321 332 508 519 522 567 570 672 1235 1552 1756
 fnu4HI (GCNGC): 2017 2024 2326 2329
 38 331 1329
 fnuDII (CGCG): 405 606 857 1068 1203 1605 1844 1857 2175
 foki (GGATG): 96 258 325 814 883 1290
 gsuI (CTGGAG): 363 524 799
 haeII (RGC GCY): 438 501 534 543 612 658 769 1366 1776 2328
 haeIII (GGCC): 295 420
 hgaI (GACGC): 484 2152 2342
 hgiAI (GWCWC): 330 364 525 800 1328
 hhaI (CGGC): 330 364 525 800 1328
 hinPI (GCGC):

hincII (GTYRAC) : 2348
 hindII (GTYRAC) : 2348
 hinfI (GANTC) : 204 451 585 914 1120 1148 1275 1500 1829 2070 2407
 hinfI (GRCGYC) : 25
 hpaII (CCGG) : 139 361 684 996 1227 1239 1602 2128 2354 2367 2372
 hphI (GGTGA) : 3 181 346 1023 1434 1832
 hpy188I (TCNGA) : 51 79 252 476 491 582 806 946 1568 1809 1814
 hpy188III (TCNNGA) : 97 281 402 443 1051 1074 1209 1289 1446 1873 1933 2156 2366
 hpy99I (CGWCG) : 27 2347
 hpyCH4III (ACNGT) : 643 1354 1573
 hpyCH4IV (ACGT) : 26 43 149 668
 hpyCH4V (TGCA) : 34 416 521 671 1030 1283 1524 1995 2023 2051 2104 2380
 kpnI (GGTACC) : 1295 2374
 ksp632I (CTCTTCNNNN) : 15 487 862 1100 1177
 maeI (CTAG) : 243 1210 1216 1396 1504 1805 1849 1889 2140 2337
 maeII (ACGT) : 26 43 149 668
 maeIII (GTNAC) : 4 180 1435 2158
 mboI (GATC) : 271 628 786 823 960 1090 1320 1566 1599 1610 1644 1812 1817 1937
 2183
 mboII (GAAGA) : 15 131 380 488 588 825 862 917 1101 1177 1219 1450
 mcrI (CGRYCG) : 755 2327
 mfeI (CAATTG) : 1622
 mluI (ACGGGT) : 37
 mlyI (GAGTCNNNNN) : 204 451 585 1120 1500 2407
 mnII (CCTC) : 65 77 126 185 209 227 246 344 350 396 469 545 562 598 724 749 853
 865 886 1021 1168 1180 1270 1287 1293 1324 1402 1738 1835 2005 2146
 2366
 mroI (TCCGGA) : 437
 msci (TGGCCA) : 175 1788 1915 1981 2220 2361
 mseI (TTAA) : 400 1405 1407
 mslI (CAYNNNNRTG) :

mspAI (CMGCKG) :	568 1672
mspi (CCGG) :	139 361 684 996 1227 1239 1602 2128 2354 2367 2372
munI (CAATTG) :	1622
mvaI (CCWGG) :	528 609 813 882 1038 1113 1137 1144 1342 1363 1638 2061
mvnI (CGCG) :	38 331 1329
mwol (GCNNNNNNNGC) :	303 312 315 321 357 502 535 641 650 793 802 1555 1665
nciI (CCSGG) :	139 360 684 995 996 1239 1602 2353 2354
ndeII (GATC) :	271 628 786 823 960 1090 1320 1566 1599 1610 1644 1812 1817 1937
	2183
nlaiII (CATG) :	32 199 336 555 1014 1075 1315 1407 1497
nlaiV (GGNNCC) :	270 532 533 558 640 705 991 1054 1140 1164 1295 1609 1741 1985 2374
notI (GCGGCCGC) :	2326
nspBII (CMGCKG) :	568 1672
nspHI (RCATGY) :	31 335
nspi (RCATGY) :	31 335
paer7I (CTCGAG) :	62
paiI (GGCC) :	438 501 534 543 612 658 769 1366 1776 2328
pflfI (GACNNNGTC) :	451
pleI (GAGTCNNNN) :	204 451 585 1120 1500 2407
ppuMI (RGGWCCY) :	558 1984 2142
pshAI (GACNNNGTC) :	553
pspAI (CCCGGG) :	995 2353
pspGI (CCWGG) :	528 609 813 882 1038 1113 1137 1144 1342 1363 1638 2061
pspOMI (GGGCCC) :	533
psti (CTGCAG) :	520 2379
pvuII (CAGCTG) :	568
rcaI (TCATGA) :	1074
rmai (CTAG) :	243 1210 1216 1396 1504 1805 1849 1889 2140 2337
rsaI (GTAC) :	41 387 1296 1897 2375 2387
rsrII (CGGWCCG) :	2368

sacI (GAGCTC) :	484 2342
sali (GTCGAC) :	2348
sapI (GCTCTTCNNNN) :	15 486 1099
sau3AI (GATC) :	271 628 786 823 960 1090 1320 1566 1599 1610 1644 1812 1817 1937 2183
sau96I (GGNCC) :	533 534 559 705 769 909 1140 1776 1985 2143 2369
sbfi (CCTGCAGG) :	2378
scrFI (CCNGG) :	139 360 528 609 684 813 882 995 996 1038 1113 1137 1144 1239 1342 1363 1602 1638 2061 2353 2354
sfaiI (GCATC) :	1067
sfcI (CTRYAG) :	10 520 2379 2400
sfii (GGCCNNNNNGGCC) :	534
smaI (CCCGGG) :	995 2353
smli (CTYRAG) :	62 2006 2147
snaBI (TACGTA) :	42
speI (ACTAGT) :	2336
sphI (GCATGC) :	31
spli (CGTACG) :	40
sse8387I (CCTGCAGG) :	2378
sspi (AATATT) :	1528 1949
ssti (GAGCTC) :	484 2342
taiI (ACGT) :	26 43 149 668
taqI (TCGA) :	63 443 1259 1322 2349
tffi (GAWTC) :	914 1148 1275 1829 2070
thai (CGCG) :	38 331 1329
tlii (CTCGAG) :	62
tru9I (TTAA) :	175 1788 1915 1981 2220 2361
tsei (GCWGC) :	292 312 315 318 321 508 519 522 567 570 672 1235 1552 1756 2017 2024
tsp45I (GTSAC) :	4 180 1435 2158
tsp509I (AATT) :	55 410 842 942 1250 1382 1623 1668 1748 1880 2107 2359 2363

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tsPRI (NNCAGTGNN) :
tth111I (GACNNNGTC) :
    1574 1821 1992 2243
    451
vspI (ATTAAT) :
    1787 2219 2360
xbaI (TCTAGA) :
    1209
xhoI (CTCGAG) :
    62
xhoII (RGATCY) :
    270 822 1609
xmaI (CCCGGG) :
    995 2353
xmaII (CGGCGG) :
    2327
xmnI (GAANNNTTC) :
    375 1159 1379 1469 2358

not found:
acII (AACGTT), afeI (ACCGCT), aflII (CTTAAG), ahdI (GACNNNNNGTC), alw44I (GTGCAC), apaI (GTGCAC), ascI (GGCGGGCC),
avaII (ATGCAT), aviI (TGGGCA), avrII (CCTAGG), baeI (NNNNNNNNNNNNNNNNNNNGTAYCNNNNNNNNNNNN), bbrPI (CACGTG),
bcgI (NNNNNNNNNNNNNNNNNNNNNNNNNNNNNN), bciVI (GTATCC), bclI (TGATCA), bfrBI (ATGCAT), bfrI (CTTAAG), blnI (CCTAGG),
blpI (GCTNAGG), bpul102I (GCTNAGC), bsaBI (GATNNNNATC), bsaxI (NNNNNNNNNNNNNNNNNNNNNNNNNNNNNN), bsiCI (TTCGAA),
bsmBI (CGTCTCNNNNN), bsmI (GAATGCN), bsp106 (ATCGAT), bsp1407I (TGTACA), bspCI (CGATCG), bspDI (ATCGAT), bsrBI (GAGCGG),
bsrDI (GCAATGNN), bsrGI (TGTACA), bsshII (GGCGGC), bst1107I (GTATAC), bstBI (TTCGAA), bsteII (GGTNACC), bstz17I (GTATAC),
bsu36I (CCTNAGG), celII (GCTNAGC), claI (ATCGAT), drdI (GACNNNNNGTC), eam1105I (GACNNNNNGTC), ecII (GGCGGA), eco47III (AGCGCT),
eco72I (CACGTG), eco81I (CCTNAGG), eheI (GGCGGC), esp3I (CGTCTC), espI (GCTNAGG), fseI (GGCGGGCC), fspI (TGGGCA), hindIII (AAGCTT),
hpaI (GTTAAC), kasI (GGCGCC), kspI (CCGCGG), mami (GATNNNNATC), mstII (CCTNAGG), naeI (GGCGGC), narI (GGCGCC), ncoI (CCATGG),
ndeI (CATATG), ngoMI (GCCGGC), nheI (GCTAGC), nruI (TGGCGA), nsiI (ATGCAT), pacI (TTAATTAA), pciI (ACATGT), pfIMI (CCANNNNNTGG),
pmeI (GTTTAAAC), pmlI (CACGTG), ppul10I (ATGCAT), psiI (TTATAA), psp1406I (AACGTT), pvuI (CGATCG), sacII (CCGCGG), sandI (GGGWCCC),
sauI (CCTNAGG), scaI (AGTACT), sceI (TAGGGATAACAGGTAAT), sexAI (ACCWGGT), sfuI (TTCGAA), sgiI (GCGATCG), sgrAI (CRCCGGYG),
snoI (GTGCAC), snoI (GTGCAC), srfI (GGCGGGC), sstII (CCGCGG), stuI (AGGCCT), styI (CCWGGG), swaI (ATTAAAT),
xcmI (CCANNNNNNNTGG)

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